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Use of the FilmArray System for Detection of *Zaire ebolavirus* in a Small Hospital in Bo, Sierra Leone

Tomasz A. Leski,[a] Rashid Ansumana,[b,c,d] Chris R. Taitt,[a] Joseph M. Lamin,[b] Umaru Bangura,[b] Joseph Lahai,[b] George Mbayo,[e] Mohamed B. Kanneh,[e] Ben Bawo,[e]† Alfred S. Bockarie,[b] Matt Scullion,[f] Cynthia L. Phillips,[f] Cynthia P. Horner,[e] Kathryn H. Jacobsen,[g] David A. Stenger[a]

Naval Research Laboratory, Washington, DC, USA[a]; Mercy Hospital Research Laboratory, Bo, Sierra Leone[b]; Njala University, Njala, Sierra Leone[c]; Liverpool School of Tropical Medicine, Liverpool, United Kingdom[d]; Mercy Hospital, Bo, Sierra Leone[e]; BioFire Defense LLC, Salt Lake City, Utah, USA[f]; George Mason University, Fairfax, Virginia, USA[g]

Laboratories associated with small hospitals often have limited expertise, personnel, and equipment to rapidly identify rare and emerging infectious diseases. We describe the successful use of the FilmArray system for rapid detection of Ebola virus directly from clinical samples in 6 out of 83 tested subjects in a small health care center in Sierra Leone.

Clinical diagnostic laboratories associated with small health care facilities often lack the personnel, resources, and expertise for rapid identification of rare or emerging infectious diseases, relying instead on reference diagnostic laboratories for specialized tests. In developing countries, especially in outbreak situations, this strategy can cause significant delays in isolation and treatment of infected (and potentially highly infectious) individuals. The ability for a small clinical lab to identify persons infected with a sporadic or epidemic pathogen, while maintaining a low logistical burden, would be of great advantage in remote or resource-limited regions, where patients with high-impact pathogens are encountered infrequently. This study describes the deployment and use of the FilmArray multiplex PCR instrument for detection of *Zaire ebolavirus* in patients and health care workers (HCWs) suspected to have Ebola virus disease (EVD) in Mercy Hospital in Bo, Sierra Leone.

Study. Mercy Hospital is a small private hospital in Bo, Sierra Leone, that refers about 2,000 patients per year to its own clinical laboratory for testing (1). Prior to the 2014 EVD outbreak, Mercy Hospital Research Laboratory (MHRL) initiated a community-based research study that included use of BioFire's FilmArray instrument and the associated BioThreat (BT) Panel in a "research-only" (rather than diagnostic) capacity. The research protocol was approved by the Sierra Leone Ethics and Scientific Review Committee (SLESRC) and by the institutional review boards of Njala University, George Mason University, and the U.S. Naval Research Laboratory. Infectious agent detection using the BT Panel is based on nested PCR (coupled with reverse transcription for RNA viruses) and melting curve analysis for amplicon discrimination (2). The BT Panel simultaneously tests for 16 pathogens, including *Zaire ebolavirus*; analysis is complete within 60 min, and the system yields a dichotomous detected/not detected result for each of the 16 targets tested. Given that this test requires only simple handling steps—dilution of whole blood and loading of the specimen into the test pouch—and an identical assay (the BioThreat-E test, limited to reporting results for *Zaire ebolavirus*) has recently received emergency use authorization (EUA) for use in the current outbreak in West Africa (3), we sought to detect the occurrence of *Zaire ebolavirus* among persons referred to the MHRL facilities for diagnostic testing and to assess the system's utility under conditions of sporadic use. No medical decisions were based on the BT Panel testing results.

In the period between 4 July 2014 and 19 January 2015, MHRL tested a total of 83 individuals (including 56 patients and 27 HCWs) using the BT Panel (see Table S1 in the supplemental material). The clinical specimens were obtained from symptomatic, suspected cases of EVD ($n = 63$) and from asymptomatic individuals with known, unprotected exposure to confirmed EVD cases ($n = 20$). All testing was performed on whole-blood specimens, with the exception of two subjects, from whom the only samples collected were a throat swab (patient 2) or urine sample (patient 3). World Health Organization (WHO)/Centers for Disease Control and Prevention (CDC) and Médecins Sans Frontières (MSF) safety guidelines were followed when handling suspected EVD patients and their clinical specimens (4, 5). All patients who met the case definition for suspected EVD and one asymptomatic patient who tested positive for *Zaire ebolavirus* by FilmArray BT Panel were immediately referred to the Bo District Health Management Team (DHMT), which initiated formal testing and case management under protocols implemented by the Sierra Leone Ministry of Health and Sanitation.

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Address correspondence to Tomasz A. Leski, tomasz.leski@nrl.navy.mil.

† Deceased.

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This paper is dedicated to our colleague, Ben Bawo, who died in service to his patients, and to his family.

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TABLE 1 Subjects testing positive for the presence of *Zaire ebolavirus* by the FilmArray BioThreat Panel or RT-PCR[a]

Ebola patient	Clinical history		FilmArray BioThreat Panel				RT-PCR confirmatory testing			
	Symptom(s)[b]	Date of onset of symptoms	Test date	Material tested	Pathogen detected	Cp value[c]	Place[d]	Test date	Ct values[e]	Outcome
1	F	10/07/14	10/06/14	Blood	*Zaire ebolavirus*	18.70	Kenema	10/10/14	26/23	Died
2	BFVW	10/14/14	10/16/14	Throat swab	*Zaire ebolavirus*	9.90	ND	ND	ND	Died
3	FKPUW	10/21/14	10/24/14	Urine	None	NA	Bo	10/27/14	25/24	Recovered
4	FNW	10/22/14	10/26/14	Blood	*Zaire ebolavirus*	16.00	Bo	10/28/14	25/24	Recovered
5	DFNVW	NA	11/01/14	Blood	*Zaire ebolavirus*	15.10	Bo	11/05/14	23/23	Died
6	CEH	11/14/14	11/21/14	Blood	*Zaire ebolavirus*	11.06	Bo	11/23/14	16/16	Died
7	AEJMSW	01/04/15	01/06/15	Blood	*Zaire ebolavirus*	10.10	Bo	01/08/15	NA	Recovered

[a] All dates are shown in the form month/day/year. ND, not done; NA, not available.
[b] Symptom codes: A, anorexia; B, difficulty breathing; C, chest pain; D, diarrhea; E, red eyes; F, fever; H, hiccup; J, joint pain; K, headache; M, muscle pain; N, nausea; P, profuse sweating; S, stomach pain; U, frequent urination; V, vomiting; W, weakness.
[c] Both crossing point (Cp) and threshold cycle (Ct) values correlate with viral loads in the sample (lower Cp and Ct values indicate higher viral load). Cp values are not reported by the commercial version FilmArray system and were obtained in collaboration with BioFire Defense.
[d] Place refers to the location where the RT-PCR confirmatory testing was done. Kenema and Bo are both in Sierra Leone.
[e] Threshold cycle (Ct) values reflect the results of two confirmatory real-time RT-PCR assays (NP/VP40) and were obtained from DHMT.

Test results. Six of the 83 tested individuals yielded positive Ebola virus results, with the first case identified on 6 October 2014 (Table 1). Five of the Ebola virus-positive patients were subsequently confirmed as infected with *Zaire ebolavirus* by the CDC mobile laboratories located in Kenema and Bo, Sierra Leone, using EUA-approved NP and VP40 real-time reverse transcription-PCR (RT-PCR) diagnostic assays (3) on independent blood samples. For the sixth Ebola virus-positive individual (patient 2) (Table 1), only a throat swab specimen was available for BT Panel testing, and the patient died, showing signs and symptoms consistent with EVD, prior to confirmatory testing. Notably, one patient (patient 1) (Table 1), an HCW, tested positive for the *Zaire ebolavirus* by BT Panel 1 day before becoming symptomatic (see the extended discussion in the supplemental material); this result was confirmed 4 days later at CDC facilities after the subject developed a fever.

The 77 patients who had negative test results with the BT Panel could be grouped into two categories. The first group, comprising 19 asymptomatic subjects exposed to confirmed EVD cases (see Table S1 in the supplemental material), did not meet the suspected EVD case definition and therefore were not tested by the CDC laboratory. The second group included 58 symptomatic subjects who were referred to the CDC laboratories for confirmatory testing. Only one individual from this group (patient 3), for whom only a urine sample was available at MHRL for testing using BT Panel, was diagnosed with EVD 3 days later upon additional testing performed on a whole-blood sample by the CDC. As far as we could verify by searching the DHMT patient database, none of the 57 remaining symptomatic subjects negative for *Zaire ebolavirus* by BT Panel tested positive for Ebola virus in diagnostic assays conducted by the CDC. In April 2015, we were able to contact all of these 57 individuals for extended follow-up. We confirmed that all these individuals are currently alive and have not tested positive for Ebola virus since the initial testing by FilmArray at MHRL.

Discussion. Testing of 83 individuals suspected of EVD or close contacts of confirmed EVD patients with BT Panel revealed six individuals positive for *Zaire ebolavirus*. While the small number of tested samples and design of the study do not allow us to draw statistically based conclusions on the diagnostic performance of the system, we observed that all five Ebola virus-positive subjects who were retested by an independent laboratory using RT-PCR were confirmed as positive.

Among the 58 symptomatic patients with negative Ebola virus test results by BT Panel, only one (patient 3) had a positive test result on confirmatory testing by RT-PCR. However, this was a special case, as the only material available for testing by BT Panel was urine and confirmatory testing was performed 3 days later using a different type of clinical specimen (whole blood). Urine has been shown in the past to be negative for Ebola viral RNA for some patients even in the advanced stages of acute phase of the EVD (6, 7). Therefore, we do not consider this result a false negative. For the remaining 57 negative cases, retrospective analysis of DHMT records indicated that none turned out positive on diagnostic testing conducted by the CDC. The possibility remained, however, that some of these individuals might have been diagnosed with EVD outside the Bo district, and this would not be reflected in the DHMT database. Face-to-face follow-up with all 57 subjects further suggests that the Ebola virus-negative results obtained with the BT Panel were correct.

There are several advantages to using the FilmArray system to screen suspected EVD cases and exposed individuals in health care settings like Mercy Hospital. The simplicity of use and lack of refrigeration requirements make the technology well suited for implementation in resource-limited settings. Its minimal sample handling requirements increase the level of safety within the lab, especially when specimens may potentially harbor highly hazardous pathogens such as Ebola virus. Although not demonstrated here for diagnostic purposes, the system's on-demand availability and simple detected/not detected readout could accelerate decision-making for isolation and referral of suspected cases, especially since the kit supports differential diagnoses. The manufacturer's list price for the reader ($39,500) and reagents ($129/test) may be prohibitive for clinical laboratories in resource-limited settings, especially in places that already have easy access to well-equipped reference diagnostic laboratories. However, the equipment may be cost-effective when used as part of a strategy to contain an outbreak of a highly virulent infection like EVD.

Wider use of point-of-care, multiplexed diagnostic technologies may facilitate earlier diagnosis, isolation, and treatment of

HCWs and others with known Ebola exposure or who are suspected to have Ebola disease based on clinical signs and symptoms. Test kits like the BT Panel (or BioThreat-E test) may be especially helpful in places lacking the facilities, personnel, and equipment to safely perform conventional clinical molecular diagnostics, enabling more effective use of personnel and resources in small testing facilities.

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